NOTE *Internal Medicine*

Molecular Cloning of Feline Hepatocyte Growth Factor (HGF) cDNA

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ABSTRACT. Hepatocyte growth factor (HGF) is a pleiotropic cytokine responsible for regeneration, development and maintenance of various organs, and growth, invasion and metastasis of tumor cells. A full-length feline HGF cDNA was cloned and sequenced by RT-PCR from cat liver. Feline HGF consists of 728 amino acid and contains α- and β-chains encoded in a single open reading frame. The predicted amino acid sequence of feline HGF showed 93.2, 93.3 and 93.3% homology with those of human, mouse and rat HGF, respectively. The putative proteolytic processing site, all cysteine residues, and four potential glycosylation sites are conserved in all species. Therefore, feline HGF is expected to have a similar three-dimensional structure to human, mouse and rat HGF.

KEY WORDS: cloning, feline, hepatocyte growth factor (HGF).

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Hepatocyte growth factor (HGF) was initially isolated from rat sera as a factor that stimulated DNA synthesis in hepatocytes in primary culture [11]. The deduced primary structure of HGF indicates that HGF is a heterodimer with a 69 kDa α-chain and a 34 kDa β-chain that are linked by a disulfide bond, and is a novel and unique growth factor [12]. HGF is now recognized to have a pleiotropic role including mitogenic, motogenic, morphogenic, angiogenic, and anti-apoptic activities [8, 23]. These activities are responsible for regeneration, development and maintenance of various organs, and growth, invasion and metastasis of tumor cells [1, 3, 6, 15, 24]. Recent studies showed that HGF is also involved in hematopoiesis, chondrogenesis, and bone remodeling [2, 19, 20]. Here, we report the cloning and sequencing of feline HGF to investigate its biological activities and possibility for therapeutic applications to some kinds of cat diseases.

mRNA was extracted from cat liver with a Fast Track mRNA Isolation Kit (Invitrogen, San Diego, CA), and reverse transcripted with a cDNA Synthesis Kit (Pharmacia, Uppsala, Sweden). The template cDNA of normal cat liver was amplified by PCR. Oligonucleotide primers were designed based on the sequences conserved among human, mouse and rat HGF cDNAs [9, 14, 16] (Table 1). Four overlapping cDNA that cover the entire coding region of feline HGF were amplified and cloned into a plasmid vector using a TA-cloning kit (Invitrogen). The nucleotide sequence of feline HGF cDNA was determined for both strands by using a Big Dye Terminator Cycle Sequencing Ready Reaction (Applied Biosystems, Foster, CA) and read by an Applied Biosystems 377 DNA sequencer.

The nucleotide sequence of feline HGF cDNA obtained in this study was 2257 bp long and contained its entire open reading frame encoding 728 amino acid residues (Genbank accession number AB046610). Alignment of the putative amino acid sequence of the feline HGF with those of human, mouse and rat counterparts is shown in Fig. 1. The predicted amino acid sequence of feline HGF showed 93.2, 93.3 and 93.3% homology with those of human, mouse and rat HGF, respectively [9, 14, 16].

Human, mouse and rat HGF are known to be synthesized as a single-chain pre-pro-HGF containing a signal peptide at approximately residue 32 of the N-terminal. The mature heterodimeric HGF consisting of α- and β-chains is produced by a proteolytic processing of the pro-HGF. Feline HGF isolated in this study also contains the signal peptide sequence at the N-terminal, the putative proteolytic processing site at residue 492 and 493 (Arg-Val), all cysteine resi-

Table 1. Sequences of oligonucleotide primers and their positions. Positions are shown as nucleotide numbers of human HGF (Genbank accession number M29145)

Name	Nucleotide positions in human HGF (n.t.)	Primer sequences
5'nOS	−86~−68	5'-CGACTGGCTCTTTTAGGCACTG-3'
5'nIR	502~523	5'-TTTCCTGTAGGTCTTTACCCCG-3'
7'S	456~478	5'-GAGTTCCATGATACCACACGAAC-3'
6R	1662~1683	5'-GCGTTTCTCATCTCCTTTTCCG-3'
6'S	1242~1263	5'-GTGGGAGAAGAACATGGAAGAC-3'
0R	2102~2122	5'-GGACAAAAATGCCAGGACGAT-3'
2106S	2005~2025	5'-TGTGAGGGAGATTATGGTGGC-3'
2314R	2190~2213	5'-GGGTGCTTCAGACACAGTTACATC-3'

Exhibit E

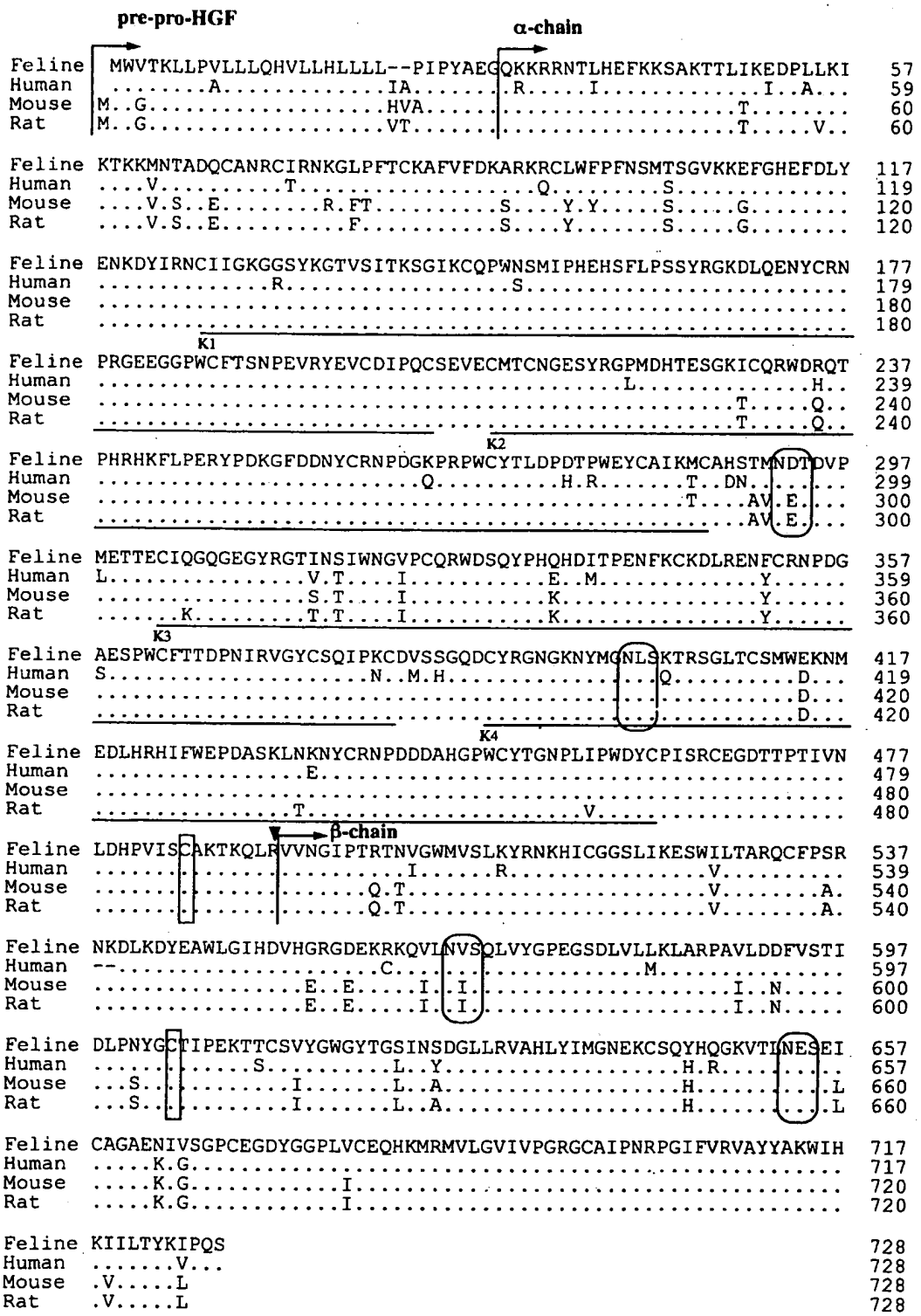

Fig. 1. Alignment of predicted feline HGF amino acid sequences with their homologues from human, mouse and rat. Identical amino acids are indicated by dots. Dashes (-) are introduced where the sequence fails. The kringle domains are shown by the underlines. The cysteine residues that form a disulfide bond between the α- and β-chains are boxed. Four putative N-glycosylation sites are circled. The cleavage site is indicated by an arrow.

dues and four potential glycosylation sites at positions equivalent to human, mouse and rat HGF (Fig. 1). From these findings, feline HGF is expected to have a similar three-dimensional structure to human, mouse and rat HGF.

Like human HGF, feline HGF has only one methionine residue at the translation initiation site, while both mouse and rat HGF have two. Additionally, feline HGF has a stop codon at the equivalent position to human HGF, and has three more residues than mouse and rat HGF at the translation termination site. Although the feline HGF sequence is two residues shorter at the signal peptide and two residues longer at the β-chain than human HGF, the total number of amino acids in human and feline pre-pro-HGF is 728.

The α-chain of HGF has four 'kringle' domains, which are also found in several proteins related to blood coagulation and fibrinolysis [12]. Previous studies showed that the N-terminal hairpin domain, the first kringle (K1) and second kringle (K2) are responsible for high-affinity binding of HGF to the c-Met/HGF receptor. The sequence of the α-chain is highly conserved among human, mouse, rat and feline HGF cDNA. In particular, the homology between the amino acid sequences in Kringle 1 of feline and human, mouse and rat are 97.5%, 100%, and 100% respectively. This high degree of conservation suggests that kringle structures in feline HGF also have the same function as in other species, and that these functions are important to the biological effects of HGF.

The β-chain of HGF alone is not able to bind the c-Met, but when c-Met is occupied by NK4, which consists of an N-terminal hairpin domain and four kringle domains, the β-chain can bind to c-Met and induce tyrosine phosphorylation [7]. The β–chain shows a high degree of homology with several serine proteases, especially that of plasmin. However, it has no serine protease activity because the histidine and serine residues of the active sites of the proteases are replaced by glutamine and tyrosine [12]. In feline HGF, these residues are also replaced, suggesting that the β-chain of feline HGF also has no serin protease activity, but rather has the activity of tyrosine phosphorylation of c-Met.

HGF has been studied for therapeutic application in various models. In lethal hepatic failure models in rats treated with D-galactosamine (D-Gal) plus lipopolysaccharide (LPS), HGF treatment prevented hepatocyte apoptosis and prevented rats from dying [5]. HGF administration also induced recovery from fatty liver in rats fed ethanol-containing diets [18]. HGF gene therapy was applied to liver cirrhosis in rats treated with dimethylnitrosamine and showed a dramatic curative effect [22]. In addition to liver diseases, HGF could be therapeutic in cases such as acute and chronic renal failure, pneumonia, cardiac infarction, hypertrophic and dilated cardiomyopathy, and gastric ulceration [4, 10, 13, 17, 21]. This study demonstrates a useful reagent for the development of a prospective cytokine therapy using HGF in diseases of cats.

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